

Alterations in the structure of the carbohydrate residues covalently associated with cell surface glycoproteins have been observed to occur during a number of biological processes, particularly during the modulation of growth, cellular transformation, and tumor metastasis. Although a number of the changes have been characterized, their significance is often unclear. An attractive but largely unsubstantiated hypothesis is that certain of these structural alterations have functional consequences. This proposed project is focused at examining the possible role of glycosylation in the regulation of the protein tyrosine kinase activity of epidermal growth factor receptor (EGF-R). The regulation of tyrosine phosphorylation by specific kinases has been shown to play critical roles in the control of cellular proliferation. The hypothesis being tested is that alterations in the structure and composition of N-linked carbohydrates covalently associated with EGF-R may play an important role in the control of cellular growth by modulating, at least in part, the protein tyrosine kinase activity of EGF-R. The demonstration of a causal structural/ functional relationship between oligosaccharide composition and enzymatic kinase activity would suggest a novel regulatory mechanism for control of EGF-R. This hypothesis is based on our previous observations that have demonstrated a dose-dependent inhibition of EGF-R's protein tyrosine kinase activity in response to exogenously added retinoic acid (RA), which was directly correlated with a dose-dependent growth inhibition of certain human glioma cells (Yung et al., 1989). Evidence also indicates that RA induces structural alterations in the glycoconjugates of EGF-R and these alterations correlate with the modulation of kinase activities (Steck et al., 1990). Furthermore, cells insensitive to the action of RA revealed no alterations in EGF-R structure or activity. The specific aims of this proposal are to: (1) Determine the structural alterations induced by RA in EGF-R; (2) Examine if a causal relationship exists between the structural alterations and functional modulation of EGF-R; (3) Analyze the biochemical mechanisms responsible for the alterations to EGF-R.

The experimental strategies to be employed are divided into structural characterizations, examinations of the structural alterations in relation to the functional modulations, and analysis of the biochemical mechanisms responsible for the structural changes. The composition of the glycoconjugates derived from EGF-R synthesized in the presence and absence of RA will be analyzed by lectin affinity chromatography, high performance anion-exchange chromatography, and analytical chemistry procedures (mass spectrometry, NMR, etc.). We are aided in these studies by previous investigations directed at the structure of EGF-R's oligosaccharides and our major interest is in determining differences between EGF-R's glycoconjugates (\pm RA) rather than analysis of all the carbohydrate structures. The demonstration of a causal relationship would entail the separation of EGF-R (+RA) from EGF-R (-RA) indicating they are independent entities and also to examine if their different structures and hence the functions of the receptors can be interconverted. The focus of the studies would then examine the biochemical mechanisms (glycotransferases or glycosidases) that are responsible for the structural changes. This proposal would, therefore, define a potential novel regulatory mechanism for control of EGF-R, along with providing compelling evidence for the possible role of carbohydrate residues in cell surface-mediated signal transduction.